# REPORT DOCUMENTATION PAGE

AFRL-SR-AR-TR-05-

0380



| 1. AGENCY USE ONLY (Leave blank) | 2. REPORT DATE | 3. REPORT TYPE AND DATES COVERED |
|---|---|---|
| | July 26, 2005 | Final Technical Report 11/01/01 to 12/31/03 |

**4. TITLE AND SUBTITLE**
Retinal processing: polarization vision in teleost fishes

**5. FUNDING NUMBERS**
F49620-01-1-~~0506~~ 0560

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**8. PERFORMING ORGANIZATION REPORT NUMBER**
N/A

**9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)**
Air Force Office of Scientific Research
875 North Randolph Street
Arlington, VA 22203

**10. SPONSORING / MONITORING AGENCY REPORT NUMBER**
N/A

**11. SUPPLEMENTARY NOTES**
N/A

**12a. DISTRIBUTION / AVAILABILITY STATEMENT**
Approved for public release; distribution unlimited

NSL

**12b. DISTRIBUTION CODE**
A

**13. ABSTRACT** ***(Maximum 200 Words)***

WHAT IS THE BASIC BIOLOGICAL UNDERSTANDING OF POLARIZATION VISION SYSTEM UNDER EXAMINATION AND HOW WE PLAN TO USE OUR UNDERSTANDING OF THIS SYSTEM TO MAKE ADVANCES IN THE AREA OF SENSOR TECHNOLOGY?

Every biological system has a model species that best illustrates structural and functional attributes of the question under examination. Teleosts fishes present an exciting opportunity to study retinal structure and function as it pertains to polarization vision. For example, salmon and coral reef fishes both display behaviors dependent on polarization perception. Our recent findings demonstrate that polarization acuity (dimensionality) is dependent on two important principles: (i) the geometry of the cone photoreceptor mosaics; (ii) the functional organization of opponency-based retinal processes. Our understanding of biological structure and processes is directed towards developing computational and silicon (VSLI) chip-based models with significant promise for the development of target detection and navigational guidance systems for aquatic, terrestrial or aeronautical autonomous vehicles.

**14. SUBJECT TERMS**
N/A

**15. NUMBER OF PAGES**
7

**16. PRICE CODE**

| 17. SECURITY CLASSIFICATION OF REPORT | 18. SECURITY CLASSIFICATION OF THIS PAGE | 19. SECURITY CLASSIFICATION OF ABSTRACT | 20. LIMITATION OF ABSTRACT |
|---|---|---|---|
| Unclassified | Unclassified | Unclassified | |

# Final Report

US AIR FORCE OFFICE OF SCIENTIFIC RESEARCH
CHEMISTRY AND LIFE SCIENCES DIRECTORATE
BIOINSPIRED PROGRAM

**RESEARCH CONCENTRATION AREA: SENSING, STIMULATION AND SIGNAL TRANSDUCTION**

Project Title: *Retinal processing: polarization vision in teleost fishes.*

**OPERATING GRANT: F49620-01-1-~~0506~~ 0560 (2001-2003)**

Program Manager: Dr. Willard Larkin

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**1. Program Overview -**

Recent research has shown that a variety of fishes possess polarization sensitivity and that this capability provides important information for guiding behaviour. Like color vision, polarization vision depends on the possession of at least two distinct receptor mechanisms, which are differentially sensitive with respect to e-vector orientation. In the case of salmonid fishes, all cone visual pigment absorption spectra overlap in the ultraviolet (UV) part of the spectrum, enabling inter-receptor comparisons, minimizing spectral confounds. Without this capability, fish would not be capable of performing discriminations of e-vector independent of brightness or hue differences (Mussi et al., *in press*). This polarization opponent behaviour, which forms the basis for e-vector coding, is exhibited by higher order neurons in the CNS. Interestingly, similar neuronal performance is evident in retinal ganglion cells where single-unit recording reveals differential responses to vertical and horizontal polarized stimuli. This clearly points to retinal circuitry as being the key candidate mediating the interaction of differentially sensitive polarization detectors.

The focus of our research has been to address the potential role that specific retinal neurons play in shaping polarization sensitivity. We have developed a plausible model for retinal processing based on input from the cone mosaic in salmonid fishes. Our working model describes the interaction of different classes of polarization detectors with retinal interneurons such as horizontal and bipolar cells. In all six species of Pacific salmonid fishes examined, there is an experimentally demonstrable linkage between the two dimensional geometry of the cone mosaic and the observed opponent polarization sensitivity (Hawryshyn, C.W. 2000. Ultraviolet polarization vision in fishes: possible mechanisms for coding e-vector. Proc. Trans. Roy. Soc.(Lond.) 335: 1187 – 1190.

The key importance of our research was to gain a framework level understanding of how the retina encodes polarization information in salmonid fishes. We established the relation between cone photoreceptors and retinal interneurons such as horizontal and bipolar cells underlying responses to polarized light. The current literature suggests that the visual system segregates the information flow into two channels: non-opponent and opponent. Direct evidence for such channels is available in the spectral, spatial and intensity domains. In our project, we used an integrated and multidisciplinary approach to reveal polarization processing by the retina. The information from this research will provide critical guideposts for electrophysiology, morphology and quantitative modeling (compartmental modelling, NEURON and parallel GENESIS – Theodore Haimberger). Such modelling approaches can provide a functional description of the polarization vision machinery that is of vital important to electro-optical engineers for designing imaging systems.

**2. Program Accomplishments and Findings -**

**A. Development of Infrastructure –**

Our research program under AFOSR BioInspired Themes has required extensive development of unique cutting-edge technology to conduct experiments on retinal processing in UV polarization vision of fishes. We have built two custom engineered electrophysiology platforms, a whole-cell patch-clamp recording rig and an intracellular recording rig (single unit, ERG, and compound action potential capability), fully integrated with high performance optical systems optimized for the 300-800 nm wavelength range and for the delivery of polarization stimuli. Proper execution of experiments requires detailed engineering design and machining with all members of the team working on different aspects of the infrastructure (software and hardware).

Our design of UV polarization optics integrated into microscopy applications is based on a vast range of experience over a period of 20 years of research in this area. This is of critical importance since most retinal electrophysiology has been restricted to the 400-700 nm wavelength range and has not included the delivery of linearly polarized stimuli, even though the species used in these studies are known to possess ultraviolet-sensitive cone photoreceptors and in some cases polarization vision. The UV spectrum (here we define UV spectrum to be that portion of the spectrum, which is visually effective - mostly $UV_A$, 300-400 nm) has been largely ignored because it is difficult and expensive to build optical systems to conduct research on UV photoreception. Secondly, much of the work on retinal electrophysiology is done with emphasis on models of human vision.

This presents an exciting opportunity for my laboratory to establish the role of UV-sensitive (UVS) cones in interneuronal processing and how UVS cones may interact with the input of other cone types in shaping color-coding. Furthermore, since UVS cones mediate polarization vision in many species, we must understand the physiological and structural patterns of connectivity of UVS cones in the vertebrate retina. Thus it was imperative to develop a laboratory with the dynamic capability of delivering the full range of optical stimuli, the most advanced electrophysiological recording platforms and software environment, to authoritatively advance knowledge in these unexplored areas of visual processing.

**B. Molecular Techniques to Examine the Expression Patterns of Photoreceptors and Other Retinal Neurons**

Kathy Veldhoen undertook a series of molecular studies to examine the expression patterns of photoreceptors and other retinal neurons in rainbow trout. Two technical approaches were used; one examining the proteome (protein expression within the retina) using ICAT proteomic analysis and a second examining the transcriptome using quantitative PCR to look at mRNA levels (representing the opsin gene expression in four distinct locations in the retina). The latter study was especially important for our electrophysiology work since we now understand opsin expression patterns (hence cone distribution) when examining UV polarization sensitivity throughout the retinal hemisphere. This research has defined the molecular components and mechanisms underlying the UV polarization neural network, and how this network responds to and is modulated by biological signal molecules such as thyroid hormone (TH).

*The Transcriptome*

We have quantified opsin gene expression using the advanced technique of real-time quantitative polymerase chain reaction (QPCR). This technique allows the highly accurate assessment of steady state mRNA transcript levels within specific target tissues under developmental regulation. By using QPCR, we examined the expression level of each opsin gene to exogenous thyroid hormones (TH) stimuli. Not only the temporal, but the spatial asymmetry of opsin gene expression across the retina was investigated. Specifically, we quantified expression of the five known rainbow trout photoreceptor opsins (SWS1 – UV-sensitive (UVS) cone opsin gene, SWS2 – short wavelength-sensitive (SWS) cone opsin gene, LWS – long wavelength-sensitive cone opsin gene, RH1- rod opsin gene, RH2- mid wavelength-sensitive (MWS) opsin gene) in control and treated retinal quadrants (DN=dorsal nasal, VN=ventral nasal, DT=dorsal temporal, VT=ventral temporal) after 2, 9 and 22 days of TH treatment.

We demonstrated that treatment with TH has significant effects, both spatially and temporally, on the transcript levels of some opsins. Most notably, SWS1 gene expression is strongly down-regulated. This observation is consistent with the results of previous studies showing that UVS cones, which express the SWS1 opsin gene, disappear in parr fish under the influence of TH. Coincident with the TH-induced decrease in SWS1 gene expression, is an increase in SWS2, RH2 and LWS gene expression. To our knowledge, this is the first demonstration that TH influences the expression levels of LWS opsin gene, however, TH has been shown influence SWS and MWS opsin gene expression.

*The Proteome*

Changes in the expression of mRNA do not always positively correlate with changes in the steady state levels of their encoded protein products. In order to determine TH dependent modulation of specific opsin proteins, we employed the ICAT (isotope-coded affinity tags) proteomic method. This novel, high throughput method uses differential isotope labelling in conjunction with mass spectrometry to measure and compare the steady state levels of proteins between two samples.

Our study directly compared the proteome of 9 day control and TH-treated whole retina. Many proteomic methods rely on two-dimensional gel electrophoresis. Membrane proteins, such as photoreceptor opsins are notoriously difficult to resolve in 2-D gels. It is noteworthy that the ICAT method was able to identify 4 of the 5 known opsins (SWS2, LWS, RH1, and RH2) in our retinal homogenates. A comparison of our QPCR and ICAT data indicates a positive correlation between changes in opsin transcript and protein levels for SWS2, RH1 and RH2 opsins following TH induction, supporting the significant role TH plays in changing the molecular components required for rainbow trout visual sensory processing, and, specifically, the UV polarization network.

In summary, using molecular techniques, we discovered factors in the retinal transcriptome and proteome involved in processes such as cell proliferation and cell death that remodel the retinal mosaic in response to physiological and environmental changes. Changes in gene expression circuitry and cellular signalling cascades form the underlying foundation of neuronal networks.

**C. Multimodal Polarization Sensitivity in Damselfish**

Using electroretinogram recording and microspectrophotometry we investigated spectral sensitivity and ultraviolet polarization sensitivity in three species of coral reef fishes commonly known as damselfishes. Here we show that three species of damselfishes (three-spot damselfish, *Dascyllus trimaculatus*; blacktail damselfish, *D. melanurus*; and blue-green chromis, *Chromis viridis*) have four classes of cone photoreceptors ($\lambda$max ranges: UVS 357-367 nm; SWS 469-478 nm; MWS 482-493 nm; LWS 512-524 nm; rods 499-500 nm). The three species shared similar combined spectral sensitivity but surprisingly complicated and varied polarization sensitivity (PS). Damselfish examined in this study have three and four channel polarization sensitivity, the most complex polarization sensitivity recorded for any vertebrate. Such capacity could play an important role in mediating a conspecific visual communication network utilizing polarized light signals in the coral reef environment.

These observations have provoked interest in the notion of dimensionality in polarization vision. Thus far, research in my laboratory has shown two (salmonid fishes), three (*Dascyllus sp.* Damselfish) and four (*Chromis viridis*, damselfish) channel polarization sensitivity (Parkyn and Hawryshyn 1999, 2000; Hawryshyn 2000; Hawryshyn et al 2003). A comparison with color vision indicates that the number of differentially sensitive receptors dictates the acuity of discrimination. In terms of polarization vision, the number of differentially sensitive detector classes determines the numerical interaction of these receptors through interneuronal networks and hence the capacity for information processing. This in turn enables the fish (to varying degrees) to discriminate one target from another and how different the targets must be, with respect to e-vector orientation, for successful discrimination. The discrimination behaviour literature for both color and polarization vision predicts that increasing the number of polarization detectors serves to reduce confusion points when making discriminations in the visual environment.

**D. Ultraviolet polarization sensitivity in rainbow trout (*Oncorhynchus mykiss*): mechanisms of retinal processing**

Ultraviolet (UV) polarization sensitivity (PS) in rainbow trout (*Oncorhynchus mykiss*) was measured using two electrophysiological methods for population recording; electroretinograms (ERG) and optic nerve compound action potential (CAP) recordings. Here we show two distinct UV PS curves: (i) one that represents ganglion cell activity (CAP) conforming to a W-shaped tuning curve with maxima at 0° and 90°, and (ii) another that represents outer retina activity (ERG b-wave) conforming a W-shaped tuning curve in addition to intermediary peaks at 45° and 135°. Using chromatic adaptation and intraocular injections of cobalt chloride, we show that the intermediary PS peaks disappear. Cobalt blocks connexin mediated gap junctions such as those used in feedforward and feedback interneuronal network processes in the outer plexiform layer that processes

polarization input. These results extend our understanding of how the retina processes polarization input to form two-channel PS system in rainbow trout. We propose a framework for the role of interneuronal processing in retinal information transfer of polarization.

**3. Personnel –**

Research Associate: Dr. James R. Plant

Research Technician/Lab Manager:
Ms. Kathy Veldhoen

Graduate Student: Ms. Leslie Anderson

**4. Peer-reviewed Publications and Conference Presentations (under AFOSR Funding)–**

**A. Gene expression work -**

Publications:

Allison, W.E., Hawryshyn, C.W. and Veldhoen, K. Thyroid hormone-dependent proteomic changes in the retina of *Oncorhynchus mykiss* (Molecular Vision, in revision).

Veldhoen, K.M., Allison, W.T., Veldhoen, N., Anholt, B.R., Helbing, C.C. & Hawryshyn, C.W. Spatio-temporal characterization of retinal opsin gene expression during thyroid hormone-induced and natural development of rainbow trout. Visual Neuroscience (submitted)

Presentations:

Veldhoen KM, WT Allison, CW Hawryshyn 2003. Proteomic analysis of retinal development using ICAT. Association for Research in Vision and Ophthalmology, Annual Meeting, Ft. Lauderdale FL, USA.

Veldhoen, K.M., Allison, W.T., Veldhoen, N., Anholt, B.R., Helbing, C.C. & Hawryshyn, C.W. 2004 Spatio-temporal characterization of retinal opsin gene expression during thyroid hormone-induced and natural development of rainbow trout. Society for Neuroscience Annual Meeting, San Diego.

**B. Electrophysiological examinations –**

Publications:

Hawryshyn, C.W., H.D. Moyer, W.T. Allison, T. von Haimberger, & W.N. McFarland. 2003. Multi-channel polarisation sensitivity in Damselfish. Journal of Comparative Physiology A 189: 213–220.

Ramsden, S., Anderson, L., Mussi, M., Kamermans, M. & Hawryshyn, C.W. Ultraviolet polarization sensitivity in rainbow trout (*Oncorhynchus mykiss*): mechanisms of retinal processing. Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology (submitted)

Presentations:

Hawryshyn, C.W., H. Moyer, W.T. Allison, T.J. Haimberger & W.N. McFarland. 2002. Polarization in coral reef fishes: Multi-channel detection capabilities in Damselfishes. Invest. Ophthalmol. Vis. Sci., 2002. 43 (4): S4548.

Ramsden, S., Anderson, L., Mussi, M., Kamermans, M. & Hawryshyn, C.W. 2004 The role of feedback in polarization sensitivity: evidence for opponent interactions. Society for Neuroscience Annual Meeting, San Diego.

## C. Other Relevant Publications (these papers not under AFOSR funding)

Degner, S. & C.W. Hawryshyn. 2001 Orientation of rainbow trout (*Oncorhynchus mykiss*) to linearly polarised light fields. Can. J. Zool. 79: 407-415

Hawryshyn, C. W., T. J. Haimberger & M. E. Deutschlander. 2001. Microspectrophotometric measurements of vertebrate photoreceptors using CCD-based detection technology. J. Exp. Biol. 204: 2401-2413.

Deutschlander, M. E., D. Greaves, T. J. Haimberger & C. W. Hawryshyn. 2001. Functional mapping of UV photosensitivity during metamorphic transitions in a salmonid fish, *Oncorhynchus mykiss*. J. Exp. Biol. 204: 2431-2438.

Hawryshyn, C.W., G. Martens, W.E. Allison & B.R. Anholt. 2003. Regeneration of ultraviolet-sensitive cones in the retinal cone mosaic of thyroxin challenged post-juvenile rainbow trout (*Oncorhynchus mykiss*). Journal of Experimental Biology 206: 2665-2673.

Parkyn, D.C., Austin, J. & C.W. Hawryshyn. 2003. Orientation of salmonids to polarized light: laboratory studies. Animal Behaviour 65: 893–904.

Allison, W.E., Dann, S.G., Helvik, J-V., Bradley, C., Moyer, H. & Hawryshyn, C.W. 2003. Ontogeny of ultraviolet-sensitive cones in the retina of rainbow trout (*Oncorhychus mykiss*). Journal of Comparative Neurology 461: 294–306

Dann, S.G., W.T. Allison, D.B. Levin and C.W. Hawryshyn. 2003. Identification of a unique transcript down-regulated in the retina of Rainbow trout (*Oncorhychus mykiss*) at smoltification. Comparative Biochemistry and Physiology Part B Biochemistry and Molecular Biology 136: 849–860

Roberts N. W., Temple S. E., Haimberger T. J., Gleeson H. F. and Hawryshyn C. W. 2004. Differences in the optical properties of vertebrate photoreceptor classes leading to axial polarization sensitivity. J. Opt. Soc. Am. A. 21: 1-11.

Dann, S.G., W.T. Allison, K. Veldhoen, T. Johnson and C.W. Hawryshyn. 2004. NF-κB and c-jun exhibit exclusive binding to the SWS1 opsin proximal promoter in rainbow trout (*Oncorhynchus mykiss*). Experimental Eye Research 78: 1015-1024

Mussi, M., Haimberger, T.J., & Hawryshyn, C.W. Behavioural discrimination of polarized light Green Chromis (*Chromis viridis*). Journal of Experimental Biology (*in press*, featured article in Inside JEB))

**5. Laboratory Exchange and Development of Technical Expertise (Kamermans/Hawryshyn labs)**

August 2001 – CW Hawryshyn visit to Kamermans' Lab

At this time, I had been notified that the AFOSR-BioInspired Themes grant had been awarded. I used this opportunity to visit Dr. Maarten Kamermans (project collaborator) in Amsterdam at the Netherlands Ophthalmic Research Institute. During this week-long meeting, we had two objectives: (i) Strategic planning for the development of infrastructure. We both felt it was important to take advantage of new technology in the marketplace such as amplifiers, microscopes optical system components etc. This aspect of the visit was invaluable and ultimately resulted in the development of cutting edge recording platforms and optical systems. We also had an intensive discussion on the specialization of my optical systems for effective delivery of ultraviolet polarized stimuli. (ii) I participated as an observer in three experiments using whole-cell patch clamp recording. This provided an organizational framework for the development of custom designed software for our recording platforms and optical systems. These two objectives were met, which greatly facilitated the development of our lab.

March 2003 – CW Hawryshyn and JR Plant visit Kamermans' Lab

With the lab built and optical systems calibrated, we visited the Kamermans' Lab to conduct two weeks of experiments. This not only allowed us to ground truth our infrastructure but it gave us valuable insight into the myriad of protocols related to whole-cell patch clamp recording, and which of these are the most important to perform and in what order. This in turn has been critical in developing the software for rapid data acquisition, effective experiment control and online analysis of data. We have mastered the surgical techniques to perform retinal slice and excised retina preparations, preparing the recording rig for experiments, search protocols for visually identified neurons, verification of stable seal of patch pipette on cell membranes and steps required for recording light evoked responses.

September 2003 – M. Kamermans visit to Hawryshyn Lab

Maarten Kamermans visited my laboratory for two weeks. The objective of the visit was to: (i) develop whole-cell patch clamp experimental protocols (first phase) for two specific experiments: cone spectral sensitivity functions and horizontal cell to photoreceptor feedback pathways. Protocols now established in software; (ii) discuss vibration optimization strategies. My laboratory is located on the third floor of a building that has a specific power spectrum of vibration frequencies. Dr. Kamermans has had extensive experience with developing electrophysiological rigs under a wide variety of scenarios. Vibration optimization was successful; (iii) discuss the software environment to ensure rapid experimental control, data acquisition and decisions concerning what is practical for online analysis without compromising overall data collection (cells are held for variable but limited periods of time). This has helped amend and refine the software in some key areas of the code; (iv) discuss pharmacological manipulations that will be needed with some of the initial experiments. We identified pharmacological methodologies for dissecting or isolating features of polarization sensitivity functions that point to important loci for neural network analysis (v) discussions of where to concentrate training efforts and evaluation of personnel performance. We decided that Leslie Anderson, a graduate student associated with the project would benefit from a three week visit to the Kamermans Lab in January, 2004. Ms. Anderson would have a whole-cell patch clamp rig to herself and the assistance of several personnel in the Kamermans Lab.

January 2004 – L. Anderson visit to Kamermans Lab

There were three main areas of focus for the study period in Amsterdam. First was for Ms. Anderson to acquire specific skills for whole-cell patch clamping of photoreceptors with the guidance of Maarten Kamermans and his postdoc. Daily access to an established patch clamp rig facilitated key steps in achieving good patch-clamp results, including a range of preferred electrode characteristics, methods to approach a cell, and use of the amplifier to maximize signal information and improvement the ability to establish patch pipette seal resistance with the cell (Gig ohm range). A second objective was to learn the use and analysis of basic protocols for current and voltage clamp recordings of patched cells. She developed and applied protocols for current/voltage tests, similar to those we have established in our lab, to assess the quality of the seal between the electrode and the cell, and the ability of the cell to provide information when stimulated with light. Some work related to the protocol to test the feedback from horizontal cells to cones was explored so that we could finalize our software for performing this experiment. Third, the constituents of the intrasol

(pipette solution) and preparation of electrode solutions for intracellular recordings in photoreceptors and horizontal cells were examined for potential differences species difference between salmonids and cyprinid fishes (goldfish commonly used in experiments in the Kamermans Lab).

**6. Honours –**

Queen's University Nomination: Tier 1 Canada Research Chair in Visual Neurobiology and Behaviour, Department of Biology, Queen's University (proposal submitted April 18, 2005 – notification Sept., 2005)

J Comp Physiol A (2003) 189: 213–220
DOI 10.1007/s00359-003-0392-4

ORIGINAL PAPER

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# Multidimensional polarization sensitivity in damselfishes

Received: 30 July 2002 / Revised: 4 December 2002 / Accepted: 20 December 2002 / Published online: 6 March 2003


**Abstract** Using electroretinogram recording and microspectrophotometry we investigated spectral sensitivity and ultraviolet polarization sensitivity in three species of coral reef fishes commonly known as damselfishes. Here we show that three species of damselfishes (three-spot damselfish, *Dascyllus trimaculatus*; blacktail damselfish, *D. melanurus*; and blue-green chromis, *Chromis viridis*) have four classes of cone photoreceptors ($\lambda_{max}$ ranges: ultraviolet 357–367 nm; short wavelength-sensitive 469–478 nm; medium wavelength-sensitive 482–493 nm; long wavelength-sensitive 512–524 nm; rods 499–500 nm). The three species shared similar combined spectral sensitivity but surprisingly complicated and varied polarization sensitivity. Damselfish examined in this study have three and four channel polarization sensitivity, the most complex polarization sensitivity recorded for any vertebrate. Such capacity could play an important role in mediating a conspecific visual communication network utilizing polarized light signals in the coral reef environment.

**Keywords** Damselfishes · Electroretinogram recording · Microspectrophotometry · Polarization sensitivity · Ultraviolet sensitivity

**Abbreviations** *ERG* electroretinogram · *UV* ultraviolet · *PS* polarization sensitivity · *LWS* long wavelength sensitive · *MWS* mid wavelength sensitive · *SWS* short wavelength sensitive · *UVS* ultraviolet sensitive

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## Introduction

Little is known about the functional capabilities of vision in coral reef fishes, and how they facilitate behavior in a visually complex tropical marine environment (McFarland and Munz 1975; McFarland 1991; Barry and Hawryshyn 1999a, 1999b). In the submarine light environment, atmospheric polarization is visible through Snell's window and visibility varies with the degree of wave action on the surface. As light passes through the water column, the dominant electric vector (e-vector) of linearly polarized light and its percentage polarization change. At depth, scattering by water molecules and reflection off non-metallic substrates produces predominantly horizontally polarized light, which masks the atmospheric polarization pattern (Novales Flamarique and Hawryshyn 1997; Cronin and Shashar 2001; Wehner 2001). Previous studies have shown that freshwater teleosts such as goldfish (Hawryshyn and McFarland 1987) and salmonids (Parkyn and Hawryshyn 1993, 2000) can detect linearly polarized light. Polarization sensitivity (PS) in these species utilizes a two-channel system with vertical and horizontal e-vector tuning, which is mediated through ultraviolet (UV) photoreception (Hawryshyn 2000). However, our knowledge of this visual attribute in marine fishes has been limited to clupeid fishes (Novales Flamarique and Hawryshyn 1998a) with other marine species including coral reef fishes as yet unexplored.

Pomacentrid fishes (damselfishes) are small, diurnal planktivores that are abundant in most shallow coral reef communities. They tend to aggregate around isolated coral heads at a depth of less than 50 m, to feed during daylight, and take shelter among the coral at night (Randall and Allen 1977). Many species exhibit synchronized movements through the water column, but tend to remain localized above a particular coral

formation. Furthermore, pomacentrids (damselfishes) exhibit stereotyped courtship and spawning activities and can be extremely territorial during spawning season (Fishelson et al. 1974). Such foraging and intraspecific communication behaviors are consistent with pomacentrid fishes having excellent vision with high cone photoreceptor densities throughout the retina (McFarland 1991).

In this study, we examined spectral and polarization sensitivity of three species of damselfishes. Microspectrophotometry was used to identify the spectral absorption of the cone photoreceptors. Electroretinogram (ERG) recording was used to record responses to spectral and polarization visual stimuli. We employed a variety of mathematical modeling approaches to describe the sensitivity data obtained in the present study.

## Materials and methods

### Animals and care

Three species of juvenile damselfish, *Dascyllus trimaculatus* (three-spot damselfish; 2–6 g, mean ± SD 4 ± 1 g body mass), *D. melanurus* (blacktail humbugs; 1–7 g, 3 ± 2 g), and *Chromis viridis* (blue-green chromis; 2–4 g, 2 ± 1 g were obtained from local aquarium suppliers (Victoria, British Columbia, Canada). Fish were housed in the Aquatic Holding Facility at the University of Victoria, British Columbia. The fish were held under a natural photoperiod (Feb–Sept 2001) in artificial seawater (salinity 33 ± 2 ppt, temperature 26 ± 2°C) prior to experimentation.

### Electroretinograms

All ERG recordings of the fish were made between 0900 and 1700 hours Pacific Time. Fish were anesthetized by immersion in metomidate (10 mg $l^{-1}$), immobilized by an intramuscular injection of Flaxedil (0.05 mg $kg^{-1}$ body weight) and further anesthetized with an intramuscular injection of metomidate (0.1 mg $g^{-1}$body weight). While in a restraining cradle, the fish was respired by aerated salt water pumped over the gills. Retinal responses to light stimuli were recorded through a glass microelectrode (10–30 μm tip, fire polished) filled with artificial seawater (40 ± 2 ppt) in contact with the cornea. A metal tungsten electrode was used as a reference and it was placed on the cranium. Electrode position was manipulated until the signal:noise ratio was optimized for every experimental fish. Retinal responses were amplified 50,000 times with a cut-off bandwidth of 0.3–100 Hz and we recorded the amplitude of the b-wave. The experimental recording apparatus has been described previously (Deutschlander et al. 2001).

The optical system consisted of two quartz/halogen light sources (150 W, Wiko) for background illumination and a Xenon short arc lamp (350 W, Ushio) for stimulus generation. Intensity and spectral content of the background light was controlled by neutral-density (ND) filters (Inconel on fused silica), and short- and long wavelength-pass interference filters (Thermo Corion), respectively. In the white (broad spectrum) background condition, the background channel contained a 3.0 ND filter and no interference filter (Fig. 1, solid line). The UV-adapting condition used two background channels, one using a white background condition, while the other contained a UV-transmitting filter (UG-11 Schott glass filter) and with 0.0 ND (Fig. 1, dashed line). Note that UG-11 filter was chosen for UV adaptation because: (1) it has very good transmission in the range 300–400 nm, (2) the infrared transmission window characteristic of this filter is well outside the range of sensitivity of the long-wavelength-sensitive (LWS) visual pigment in all three species of damselfishes used in this study.

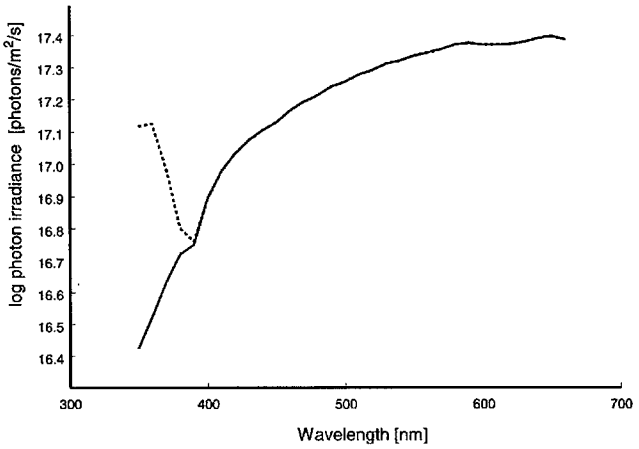


**Fig. 1** Spectral energy distribution of adapting backgrounds used in both spectral and polarization sensitivity (PS) experiments. The measurements are plotted in $\log_{10}$ photon irradiance (photons $m^{-2}$ $s^{-1}$). The *solid line* represents the white background condition and the *dashed line* represents the ultraviolet (UV) adaptation condition

A calibrated, computer-controlled stimulus delivery system was used to manipulate stimulus spectral and intensity characteristics via a ND wedge and monochrometer (Instruments SA). For spectral sensitivity experiments, a trifurcated light guide (fuse silica fibers, Fiberoptic Systems) with a quartz diffuser placed at the terminal end of the fiber optic, projected uniform depolarized illumination (background and stimulus) to the left eye of the fish. Sensitivity between 360 nm and 600 nm, in 20-nm intervals, was determined using a staggered wavelength presentation to prevent adaptation to a certain region of the spectrum. Stimulus intensity was increased in 0.2 optical density steps and stimuli were presented as 500-ms flashes for both spectral and polarization sensitivity.

PS experiments used three separate liquid light guides (Thermo Oriel), where the stimulus output terminated with a UV-transmitting linear polarizer (HNP-B Polaroid film). The light guides were positioned to superimpose the background and stimulus light on the pupil of the left eye. Confounding effects of any polarization inherent in the optics were removed through calibrating the stimulus beam's intensity at each e-vector and wavelength at the position of the test fish's eye. In a previous study, the polarization properties of the ocular media of the eye were examined in rainbow trout (Parkyn 1998) showing that percentage polarization was high (80–90%) across the visible spectrum and dropped (76%) in the UV spectrum. The decrease in percentage polarization was shown to be a result of the overall decrease in ocular media transmission in the UV portion of the spectrum. We have conducted a number of control experiments in the present study and previous work (Hawryshyn and McFarland 1987) to eliminate the ocular media as factor modulating PS (see Results for evidence which argues for the involvement of cone mechanisms in the mediation of PS).

PS was measured using ERG recording. Sensitivity was determined in either 15° or 30° increments between 0° and 180° at 360 nm. For reference, the 0°/180° e-vector axis was defined as vertical (relative to the gravitational axis) and the 90°/270° e-vector axis as horizontal (Hawryshyn and McFarland 1987). Experimental fish were light adapted to a depolarized spectrally broadband background, on which linearly polarized stimuli at a test wavelength of 360 nm with increasing intensity were presented. Thresholds were interpolated from amplitude versus intensity curves using the criterion response level of 10 μV above baseline noise amplitude (Deutschlander et al. 2001). Sensitivity was taken as the reciprocal of threshold irradiance and plotted against test e-vector orientation to give PS curves.

### Microspectrophotometry

After dark adaptation (2 h), fish were anesthetized by immersion in metomidate (50 mg $l^{-1}$) and killed by cervical transection. Hemisected eyes were maintained in physiological saline (Minimum Essential Medium solution, Sigma) in a light-tight container on ice. Samples were prepared under infrared light (880-nm Schott glass filter), by placing a sectioned piece of retina on a cover slip and teasing it apart with a razor blade. All dissections and recordings were conducted at 15°C. The experimental apparatus has been described previously (Hawryshyn et al. 2001). In brief, a calibrated measurement beam passed through a condenser lens system to form an image of a variable aperture that could be focused on the sample. The sample was positioned relative to the measurement beam using a motorized x-y stage manipulator. Infrared background illumination was used to visualize the retinal preparation. The measurement beam was comprised of broad-spectrum xenon light (150 W) passing through the specimen, which was in turn collected by a spectrograph and projected onto a back-illuminated CCD system (pixel array of 1340 columns and 400 rows; Roper Scientific). Charges that accumulate in each pixel are summed at an exit row and converted from analog potential to a digital number by an A/D converter.

The ratio of the measurement beam intensity (taken through the outer segment of the cone photoreceptor) and reference beam intensity (taken through a tissue-free area) was used to calculate spectral transmission and in turn spectral absorbance ($\log_{10} T^{-1}$) of the photoreceptor. Reference measurements were taken frequently. The exposure time for the measurement and reference beam flashes was 1,600 ms, while 60- to 120-s exposures to full spectrum and intensity was used for photoreceptor bleaching experiments. Bleaching experiments were used to confirm the identity of cone photoreceptors. Spectra were accepted based on the presence of a long-wavelength limb baseline and a good fit of an eighth-order polynomial template for $A_1$-based visual pigments (Hawryshyn et al. 2001) and template for $A_1$-based visual pigments based on Govardovskii et al. (2000). The Govardovskii template fit revealed the $\lambda_{max}$ for each spectrum, and the absorbance spectra, for each cone class, was later normalized and averaged. The averaged curve was smoothed using a 31-point boxcar smoothing function (31 points correspond to slightly less than 2 nm).

### Analysis of spectral and polarization sensitivity curves

A linear-additive model, based on the calculated $\lambda_{max}$ values, was fit to the spectral sensitivity data to represent the relative cone mechanism contributions to the recorded functions. The following formula represents the linear additive (color) model:

$$ss = k_1 * UVS + k_2 * SWS + k_3 * MWS + k_4 * LWS \quad (1)$$

where ss is spectral sensitivity, UVS, SWS, MWS and LWS are the respective visual pigment absorbance curves and $k_i$ their weight coefficients (see Table 1 for $k$ coefficients).

**Table 1** $k$ coefficients used inmodeling the spectral sensitivity of damselfishes

| $k$ value | *Chromis viridis* | *Dascyllus melanurus* | *Dascyllus trimaculatus* |
|---|---|---|---|
| $k_1$ | 0.6 | 4 | 12 |
| $k_2$ | 0.9 | 0.8 | 1 |
| $k_3$ | 0.6 | 4 | 0.4 |
| $k_4$ | 0.5 | 0.2 | 0.2 |
| $k_5$ | 1 | 1 | 0.8 |
| $k_6$ | 6 | 80 | 1800 |
| $k_7$ | 0.2 | 1 | 1 |
| $k_8$ | 0.8 | 4 | 40 |

The linear subtractive model is represented by

$$ss = k_1 * UVS + k_3 * MWS + (k_2 * SWS - k_4 * LWS) + (k_4 * LWS - k_2 * SWS) \quad (2)$$

where an LWS-SWS cone mechanism opponency has been assumed for damselfish.

For the data presented we assumed that both linear additive and linear subtractive contributions were at play to shape spectral sensitivity. The formula used was:

$$ss = k_1 * UVS + k_3 * MWS + k_5 * (k_2 * SWS - k_4 * LWS) + k_6 * (k_4 * LWS - k_2 * SWS) + k_7 * SWS + k_8 * LWS \quad (3)$$

This equation provided reasonable $k$ coefficients (see Table 1) and a good fit curve for *C. viridis* and *D. melanurus*, while *D. trimaculatus* data was harder to fit. This we attribute to possible non-linear behavior of the retinal neural network.

The PS is a periodic function of the polarization angle. A trigonometric polynomial was used to fit circular-linear regression lines to the PS data (this approach has been used extensively in Parkyn and Hawryshyn 2000):

$$ps = M + \sum_{i=1}^{n} (A_i * \cos(2 * i * \theta - \varphi_i)) \quad (4)$$

where $ps$ is the PS, $M$ the mesor (mean), $\theta$ the polarization angle, and $\phi$ the phase angle (in radians). The minimum $n$ needed to fit the data depends on the number of undulations in the data. *C. viridis* PS curve had four cycles, which means that a cosine function with $n$ equal to or greater than 4 was needed. The same formula was used for all three fits, that is, the cosine functions with $n = 1, 2, 3, 4$ were included. Since fewer data points were measured for *Dascyllus*, their data was resampled by linear interpolation for the fit. The SVDC (Singular Value Decomposition) routine prepared the coefficient matrix and the SVSOL (back-substitution) routine performed the linear least-square fit in calculating the coefficients of the fit curves. These routines are part of IDL (Interactive Data Language, version 5.3, Research Systems, Boulder, Colo., USA) used in this analysis.

## Results

Our study used microspectrophotometry (MSP) to examine the spectral absorbance properties of double and single cone photoreceptors, in all three species of damselfishes. We identified four spectrally distinct cone types for each species (Table 2, Fig. 2, panels A–O), with results consistent with a previous study where *D. trimaculatus* was examined (McFarland and Loew 1994). Of notable significance was the presence of a UV-sensitive cone type in all three species examined. Our MSP analysis was aimed at providing absorbance spectra for the various cone types that could be used to model the spectral sensitivity of all three species of damselfish.

Spectral sensitivity measurements were performed using ERG recording techniques. These experiments revealed the expression of all cones types determined using a spectrally broadband background. Figure 3, panels A, C, E shows the spectral sensitivity of the three species of damselfish used in this study. The solid line fitted to the spectral sensitivity points represents a model that takes into consideration both linear additive and subtractive processes of the cone mechanisms (see Materials and methods for the mathematical description of the model used for curve fitting). To confirm that shortwave sensitivity was mediated by the UV-sensitive mechanism, we performed UV chromatic adaptation,

**Table 2** Mean visualpigment peak absorbance values ($\lambda_{max}$) from three species of pomacentrids (damselfishes).Values are mean $\lambda_{max}$ (nm) ± 1SD with sample size (*n*). Individual curves were accepted based on the presence of a long-wavelength limb baseline and agoodness-of-fit to an eighth-order polynomial and Govardovskii et al. (2000) template based on an $A_1$ visual pigment (*MWS* medium-wavelength-sensitive, *LWS* long-wavelength-sensitive, *SWS* short-wavelength-sensitive, *UVS* ultraviolet-sensitive)

| Species | Double cones | Single cones | Rods |
|---|---|---|---|
| *Dascyllus melanurus* | 469 ± 3.6 (3) (SWS) | 357 (1) (UVS) | 500 ± 6.0 (11) |
| | 520 ± 7.7 (10) (LWS) | 482 ± 2.8 (5) (MWS) | – |
| *Dascyllus trimaculatus* | 471 ± 8.4 (9) (SWS) | 368 ± 4.2 (2) (UVS) | 499 ± 1.2 (3) |
| | 512 ± 5.1 (13) (LWS) | 485 ± 6.1 (13) (MWS) | – |
| *Chromis viridis* | 478 (1) (SWS) | 367 ± 8.0 (4) (UVS) | 499 ± 4.2 (7) |
| | 524 ± 3.5 (8) (LWS) | 493 ± 3.8 (11) (MWS) | – |

re-measured spectral sensitivity and calculated a difference spectrum for each species (Fig. 3, panels B, D, F). The difference spectra were then fitted with the UV-sensitive cone absorbance spectrum derived from the MSP measurements and in each case the correspondence of the difference curve and cone absorbance spectrum confirms the identity of a UV-sensitive cone mechanism.

Figure 4, panels A–C show PS functions that were described by a best fit using a cosine-based periodic regression model (Parkyn and Hawryshyn 2000). For the purposes of this paper, we report only those results relating to the ON response. All three species showed modulated sensitivity with respect to the orientation of linearly polarized light and the nature of this sensitivity was more complex than the two-channel "W-function" commonly observed in salmonids and cyprinids (Hawryshyn and McFarland 1987; Coughlin and Hawryshyn 1995; Novales Flamarique and Hawryshyn 1997; Parkyn and Hawryshyn 2000). For example, blacktail humbugs (*D. melanurus*) and three-spot damselfish (*D. trimaculatus*) exhibited PS with a 60°

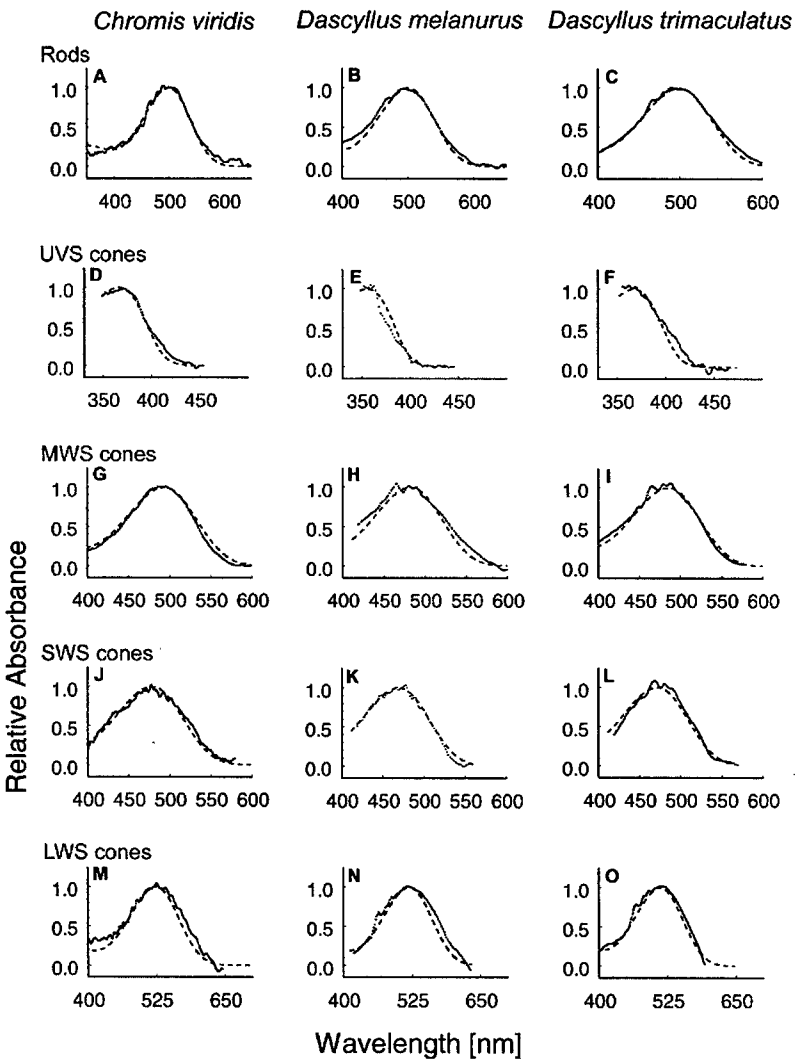


**Fig. 2** Mean visual pigment absorbance spectra for three species of damselfish. Each *column* shows the data for a given species and each *row* shows the data for a given photoreceptor type. The *solid line* represents the mean absorbance values (see Table 2 for details on sample size for each plot) and the *dashed line* represents the $A_1$ visual pigment template (Govardovskii et al. 2000). *Panels a, b, c* rods; *panels d, e, f* UV-sensitive (UVS) cones; *panels g, h, i* single medium-wavelength-sensitive (MWS) cones; *panels j, k, l* double short-wavelength-sensitive (SWS) cones; *panels m, n, o* double long-wavelength-sensitive (LWS) cones

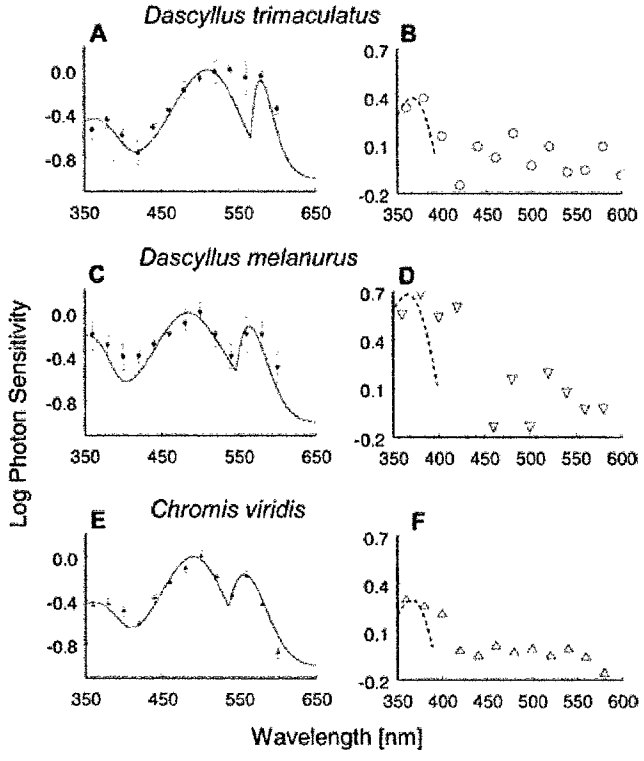


**Fig. 3** Mean spectral sensitivity of three species of damselfish. Log relative photon sensitivity ($\log_{10}$ photons $cm^{-2}$ $s^{-1})^{-1}$ was determined using the ON-responses of electroretinogram recordings to spectral stimuli under white background conditions. *Panel A*: *Dascyllus trimaculatus* ($n=3$); *panel C*: *D. melanurus* ($n=6$); *panel E*: *Chromis viridis* ($n=3$). Values represent mean sensitivity ± 1 standard error and the individual spectral sensitivity curves were normalized to 460 nm. The *solid line* fitted to the spectral sensitivity points represents a model that takes into consideration both linear additive and subtractive processes of the cone mechanisms (see Materials and methods for the mathematical description of the model used for curve fitting). Difference spectra resulting from UV chromatic adaptation are shown for *D. trimaculatus* ($n=1$) (*panel B*); *D. melanurus* ($n=1$) (*panel D*); *C. viridis* ($n=1$) (*panel F*). Each curve was generated for one individual for each species and represents the difference between the spectral sensitivity determined using a white background condition and the spectral sensitivity determined using the white background condition plus the addition of the UV-adapting background. The *dashed line* fitted to each difference spectrum represents the absorbance spectrum of the UV-sensitive cones; $\lambda_{max}$ values: 357 nm, 368 nm, 367 nm for *panels B, D, F*, respectively

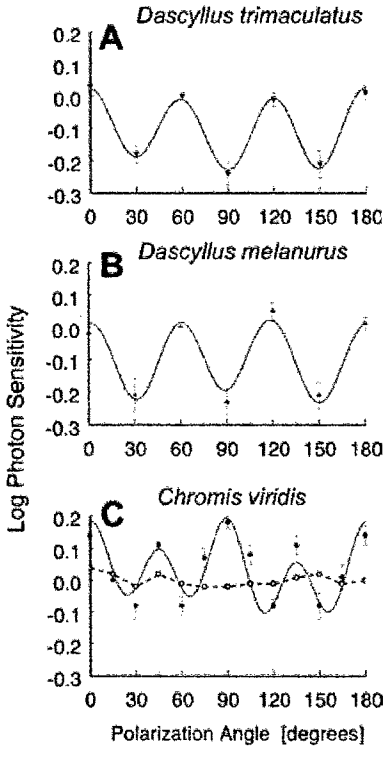


**Fig. 4** Mean polarization sensitivity of three species of damselfish. Log relative photon sensitivity ($\log_{10}$ photons $cm^{-2}$ $s^{-1})^{-1}$ was determined under white background conditions relative using the ON-responses of electroretinogram recordings to a UV stimulus 360 nm, which were plane polarized and presented at varying e-vector orientations. *Panel A*: *D. trimaculatus* ($n=3$); *panel B*: *D. melanurus* ($n=5$); *panel C*: *C. viridis* ($n=5$). Values represent mean sensitivity ± 1SE and points are normalized to 60° for *Dascyllus* spp. and 30° for *C. viridis*. The *solid line* represents a cosine-based, periodic regression of the data (see Materials and methods for mathematical description). The *dashed line* in *panel C* represents polarization sensitivity under UV adaptation

periodicity and approximately 0.3 log unit depth of modulation with sensitivity peaks at 0°, 60°, and 120° (Fig. 4, panels A, B). Blue-green damselfish (*C. viridis*) had a more complex PS function with 45° periodicity and maximal sensitivities at 0°, 45°, 90°, and 135° (Fig. 4, panel C). The low error associated with the sensitivity measurements in Fig. 4, the negative control experiment using depolarized stimuli showing no e-vector modulation (data not shown – one experiment performed on *C. viridis*; see Hawryshyn and McFarland 1987 for rationale of control experiment), and the substantive differences between genera, convincingly argues that the PS functions were non-random. Moreover, Fig. 4, panel C (open circle and dashed line) shows the results of a UV-sensitive cone mechanism adaptation experiment ($n=1$), which abolishes PS in comparison with non-adaptation condition (filled circle and solid line). This has important implications regarding the class(es) of cone mechanism(s) mediating PS in damselfishes.

## Discussion

Chromatic sensitivity of diurnal coral reef fishes is very consistent between multiple taxa, and appears to correlate well with the photic environment and how the visual system guides behavior. Shallow tropical marine waters are characterized by high spectral complexity and intense solar radiation, particularly in the UV and violet components of the spectrum relative to other wavelengths. Shallow-water fishes, including pomacentrids (damselfishes), possess short-wavelength-shifted cone opsins with spectral sensitivity being compressed to below 560 nm (McFarland 1991). The SWS, MWS, and

LWS cones of pomacentrids absorb in the range where tropical marine water is the most transparent, between 460 nm and 560 nm (Barry and Hawryshyn 1999a). As discrimination is the potentially most acute where absorbance spectra of two pigments overlap (Jacobs 1981), the three closely clustered pigments may provide pomacentrids with excellent color discrimination, as well as possible high-level contrast enhancement due to offset UV-sensitive cone sensitivity. These capabilities are likely to facilitate the visually mediated behaviors of pomacentrids such as predation on small zooplankton and algae, predator detection and avoidance, schooling, courtship, territorial defense, or other visual communication.

The absence of chromatic sensitivity to the long-wavelength part of the spectrum (longer than 600 nm) in diurnal coral reef fishes has not been adequately explained, as there is an abundance of red light in shallow marine waters. Perhaps this is an evolutionary trade-off to allow for a UV-sensitive cone mechanism in the retina, while maintaining excellent discrimination capabilities throughout the middle and lower ends of the spectrum. The presence of UV-sensitive cones in many disparate groups of fishes testifies to its importance in visual tasks, especially since its stimulation requires the retina be exposed to damage from high energy photons (Siebeck and Marshall 2001). Most coral reef fishes produce structural colors with significant UV reflection. Therefore, a UV-sensitive photoreceptor would add an extra dimension to color vision as all fish have colors with and without UV information (Losey et al. 1999). Besides contributing to wavelength discrimination, the UV-sensitive cone appears to be instrumental in polarization vision systems (Parkyn and Hawryshyn 1993, 2000; Novales Flamarique et al. 1998; Hawryshyn 2000). However, UV photosensitivity does not guarantee the possession of PS (Novales Flamarique and Hawryshyn 1998b).

Detection of visual signals from the environment depends on light transmission to and within the eye, photoreception by the retina, and a neural pathway between the retina and higher visual centers within the brain. Although MSP data provide opsin absorbance characteristics, it does not determine which wavelengths of light are physiologically relevant signals because it offers no information about the interaction of cone mechanisms through interneuronal processing and it cannot reveal the presence of a polarization vision system in the retina (Hawryshyn 2000). Electroretinographic analysis provided a unique opportunity to investigate the neuronal processing of chromatic and e-vector visual signals. The majority of past studies used compound action potential (CAP) recordings or single unit recordings to measure retinal responses at the level of the ganglion cell or higher in the visual neural pathway (Waterman and Aoki 1974; Parkyn and Hawryshyn 1993; Coughlin and Hawryshyn 1994, 1995). ERGs record the complex field potential produced by the ON-bipolar cells in the retina, with minimal input from third-order neurons (Stockton and Slaughter 1989; Dong and Hare 2000). Because we have demonstrated that complex sensitivity functions are already measurable at the level of the bipolar cell, it is likely that mediation of these signals occurs at the level of the horizontal cells and their interaction with bipolar cells. Horizontal cells form a neural network between bipolar cells and are important for comparing input from multiple photoreceptors (Stockton and Slaughter 1989). Therefore, in addition to mediating chromatic opponency, this is the first evidence that they potentially contribute to e-vector coding in a way consistent with our current model (Hawryshyn 2000). Signals from photoreceptors tuned to different e-vectors of light could be processed by horizontal cells to result in the complex PS functions measurable at the level of the bipolar cell. Unlike atmospheric signals, light underwater is slightly less polarized below 400 nm than at longer wavelengths. Seliger et al. (1994) argues that ultraviolet polarization detector systems evolved to maximize signal-to-noise considerations under lower degrees of polarization. Furthermore, Pomozi et al. (2001) have recently shown that e-vector patterns under cloudy conditions are more reliable in the ultraviolet portion of the spectrum and thus animals using ultraviolet PS have the advantage of maintaining polarization vision under variable sky vault conditions.

The complexity of pomacentrid PS suggests that both the geometry of cone mosaics and the underlying interneuronal processing of e-vector give rise to tri- and/or tetramodal PS functions. In the *Dascyllus* species, these receptors would be maximally sensitive to 0/180°, 60°, and 120°. In *C. viridis,* receptors tuned to 0/180°, and 90° are likely, while the sensitivity seen at 45° and 135° could result from either a third polarization-sensitive cone mechanism, or a non-polarization-sensitive cone mechanism released from inhibition when the other two detectors are at their minima.

Our previous research on salmonids has established that polarization vision is mediated by UV photoreception. PS depends on a well-defined square cone mosaic pattern and the biophysical properties of the square cone mosaic to some degree. The biophysical mechanism appears to be based on the selective reflection of axial polarized light by the partitioning membrane, formed along the contact zone between the members of the double cone, onto neighboring UV-sensitive cones (Novales Flamarique et al. 1998). We are currently examining the geometry of the cone mosaic in damselfishes to explore its implications for dimensionality in PS. We have currently initiated a large-scale study of the cone mosaic pattern in these species of damselfishes. However, it is important to emphasize that while the cone mosaic geometry can give us clues about the basis for PS, it is equally important to consider the role of retinal interneuronal processing in shaping the patterns of sensitivity we see in these species. In this study, we show that the UV-sensitive cone mechanism possibly plays a critical role in mediating PS in *C. viridis* by abolishing

PS with UV chromatic adaptation (Fig. 4, panel C, open circles and dashed line, $n=1$). Therefore, the relative stimulation of the UV cone maybe the signal, to which other cone responses are compared, and thus control the shape of the e-vector tuning curve.

Our study provokes the question of how dimensionality in PS can determine the utility of polarization vision in fishes. A single vertically sensitive PS channel substantially reduces background scatter, and increases contrast as well as visual range (Wehner 2001). Two-channel PS systems enable more complex behaviors mediated by polarization vision. For instance, salmonids (Hawryshyn et al. 1990; Parkyn et al. 2003), like many insects and a wide range of other invertebrates (Wehner 2001), use celestial polarized light cues as a navigational mechanism. Furthermore, evidence from cephalopods suggests that polarization vision functions such as detection of transparent or reflective prey (Shashar et al. 1998, 2000) as well as a communication channel for interspecific and intraspecific interactions (Shashar and Cronin 1996; Shashar et al. 1996). Two-channel PS systems are known to have neutral points and confusion states (Bernard and Wehner 1977), and thus are associated with coarse e-vector discrimination, possibly distinguishing 0° from 90° (Degner and Hawryshyn 2001; Hawryshyn 2000). However, experiments with octopus show that individuals can discriminate e-vector angular differences as low as 20° within a single target, possibly facilitated by head or eye movements (Shashar and Cronin 1996).

Theoretically, three-channel PS systems eliminate problems inherent with a two-channel system and afford the additional capability of assessing percentage polarization, independently of e-vector and intensity (Bernard and Wehner 1977). Therefore, the tri-modal PS observed in the blacktail and three-spot damselfish suggests very fine e-vector discrimination abilities, potentially coupled with the ability to extract percentage polarization information in a visual scene. The tetra-modal PS observed in the blue-green damselfish has e-vector discrimination capabilities, which are possibly better than in the *Dascyllus* spp., with little or no confusion states between signals of differing intensity, e-vector, or percentage polarization. An analogy can be drawn from color vision where the number of receptor mechanism classes generally correlates with wavelength discrimination capacity. Greater overlap in the sensitivity of differentially sensitive polarization detectors incurs added acuity with respect to e-vector discriminations since computations of angular disparity are based on a greater number of comparisons at a given e-vector orientation. Such complex polarization vision systems exist in bees and ants, where they are used to transmit information between individuals about the direction of food sources (Wehner 2001). Multi-channel polarization vision in mantis shrimp may play an important role in intraspecific communication (Marshall et al. 1999).

Polarization vision in pomacentrids is the most complex recorded for a vertebrate, with three- or four-channel systems that theoretically eliminate the confusion states inherent in two-channel systems, and allow for the assessment of percentage polarization. Such acute detection systems suggest that polarization vision plays an integral role in inter- or intraspecific interactions, species-environment interactions and foraging. Reflective surfaces such as a fish's body would provide an ensemble of polarization patterns to a moving animal. Since iridophores are responsible for rapid changes in coloration (Kasukawa et al. 1986; Kasukawa and Oshima 1987; Oshima et al. 2001) and possibly polarization patterns, the dermis of pomacentrids may produce both coloration and polarization used for interspecific and/or intraspecific communication.

**Acknowledgements** We thank Dr. Jim Plant and Ms. Kathy Veldhoen for comments on the manuscript. HDM was supported by an NSERC Undergraduate Research Award and WTA was supported by an Alzheimer Society/CIHR Doctoral Fellowship. This research was sponsored by the Natural Sciences and Engineering Research Council of Canada (Operating and Equipment Grants) and the Air Force Office of Scientific Research, USAF under grant number F49620-01-0560. The U.S. Government is authorized to reproduce and distribute reprints for Governmental purposes notwithstanding any copyright notation thereon. The views and conclusions contained herein are those of the authors and should not be interpreted as necessarily representing the official policies or endorsements, either expressed or implied, of the Air Force Office of Scientific Research or the U.S. Government. The experiments described in this paper comply with the "Principles of animal care", publication no. 86-23, revised 1985 of the National Institute of Health and also with the University of Victoria, Animal Care Committee under the auspices of the Canadian Council on Animal Care.

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